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Improvement of Postreceptor Events by Metoprolol Treatment in Patients With Chronic Heart Failure

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Objectives. **This study tested the hypothesis that metoprolol restores the reduction of the inotropic effect of the cyclic adenosine monophosphate (cAMP)-phosphodiesterase inhibitor milrinone, which is cAMP dependent but beta-adrenoceptor independent.**

Background. **Treatment with beta-adrenergic blocking agents has been shown to lessen symptoms and improve submaximal exercise performance and left ventricular ejection fraction in patients with heart failure. Restoration of the number of downregulated beta-adrenoceptors has been suggested to be one mechanism of beta-blocker effectiveness. However, the reversal of postreceptor events, namely, an increase in inhibitory G-protein alpha-subunit concentrations, could also play a role.**

Methods. **Fifteen patients with heart failure due to dilated cardiomyopathy (left ventricular ejection fraction 24.6 ± 1.5% [mean ± SD], New York Heart Association functional class II or III) were treated with metoprolol (maximal dose 50 mg three times daily) for 6 months. Before and after metoprolol treatment, inotropic responses to milrinone (5 to 10 μg/kg body weight per min) were measured echocardiographically. For comparison, responses to milrinone were determined under control conditions and after accelerated application of 150 mg of metoprolol to inactivate beta-adrenoceptors in subjects with normal left ventricular function.**

Results. **In subjects with normal left ventricular function, treatment with metoprolol did not alter the increase in fractional shortening or pressure/dimension ratio of circumferential fiber shortening after application of milrinone. In patients with heart failure, treatment with metoprolol significantly increased left ventricular ejection fraction, fractional shortening and submaximal exercise tolerance and reduced heart rate, plasma norepinephrine concentrations and functional class. After metoprolol treatment, milrinone increased fractional shortening but had no effect before beta-blocker treatment.**

Conclusions. **Milrinone increases inotropic performance independently of beta-adrenoceptors in vivo. Metoprolol treatment restores the blunted inotropic response to milrinone in patients with heart failure, indicating that postreceptor events (e.g., increase in inhibitory G-proteins) are favorably influenced. This mechanism could contribute to the beneficial effects observed in the study patients and represents an important mechanism of how beta-blocker treatment influences the performance of the failing heart.**

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Desensitization of beta-adrenoceptor–adenylyl cyclase signal transduction is recognized as an important mechanism contributing to impaired contractility in the failing human heart (1–3). $Beta_1$-adrenoceptors are downregulated, leading to impaired positive inotropic effects of beta-adrenoceptor agonists (1–3). However, the effects of cyclic adenosine monophosphate (cAMP)–phosphodiesterase inhibitors, which bypass beta-adrenoceptors and inhibit the breakdown of cAMP, are also impaired (4,5) and are due to postreceptor alterations in the failing heart (2). This alteration has been identified as an increase in inhibitory G-protein alpha-subunits (Giα) (6–8). Experimental studies have shown (9,10) that both the reduction of beta-adrenoceptors and the increase of Giα are induced by catecholamines. Therefore, a reversal of these defects could contribute to a functional improvement of the failing heart.

Long-term beta-adrenergic blocking agent treatment has been shown (11–13) to be beneficial in patients with heart failure due to dilated cardiomyopathy in terms of improving left ventricular ejection fraction, submaximal exercise tolerance (11–13) and, possibly, prognosis (11,14). Treatment with metoprolol has been shown (15,16) to increase the number of beta-adrenoceptors, correlating to an improvement in left ventricular ejection fraction. However, treatment with the vasodilating beta-blocker carvedilol did not increase beta-adrenoceptors despite a similar increase in left ventricular ejection fraction. Therefore, it is likely that improvement of postreceptor defects is also an important contributor to improved cardiac function.

In the present study, we took advantage of the beta-adrenoceptor–independent but cAMP-dependent positive inotropic effects of milrinone in healthy volunteers (normal left

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Abbreviations and Acronyms

ANOVA = analysis of variance
cAMP = cyclic adenosine monophosphate
Giα = inhibitory G-protein alpha-subunit
MDC = Metoprolol in Dilated Cardiomyopathy
P/D = pressure/dimension ratio

ventricular function) and in patients with moderate heart failure (left ventricular ejection fraction 25% to 42%). To investigate the effects of beta-blocker treatment on postreceptor mechanisms, we studied echocardiographic variables of contractility before and after metoprolol treatment for 6 months.

Methods

Patients. Patients with chronic, stable left-sided heart failure due to dilated cardiomyopathy (13 men, 2 women; mean [±SD] age 54.9 ± 2.1 years, range 42 to 72 years) were investigated. All patients had dilated cardiomyopathy. Significant coronary artery disease was excluded by coronary angiography in all patients. In addition, seven male volunteers (mean age 32.4 ± 4 years, range 21 to 44) were investigated as healthy control subjects.

Measurement of contractility in vivo by echocardiography. Two-dimensionally guided M-mode echocardiography in the short-axis view was performed transthoracically using a mechanical sector scanner (Vingmed CFM 750, Sonotron) with a 3.25-MHz transducer. All recordings were made by the same investigator (A.S.) and were evaluated independently by two principal investigators (H.D., M.B.) from the echocardiography laboratory. End-diastolic diameter (EDD) and end-systolic left ventricular diameter (ESD) were measured as the mean of five cardiac cycles according to standard guidelines (17). Fractional shortening was calculated as (EDD − ESD)/EDD × 100. To control for effects of potentially different afterload conditions, the pressure/dimension ratio (P/D) was calculated as the ratio of systolic blood pressure to end-diastolic dimension. In previous studies, intraobserver variability was <3% (18), and interobserver variability did not exceed 4.6% (19). The latter data fit well with those previously reported for reproducibility guidelines for M-mode echocardiography (20,21). Systolic, diastolic and mean arterial blood pressures were measured in the right arm by an automated sphygmomanometer (Critikon, Dinamap). Heart rate was obtained from simultaneous electrocardiographic recordings.

Study protocol. Echocardiographic investigations were performed before and after 6 months of metoprolol therapy in patients with heart failure. Therapy was started with a dose of 10 mg of metoprolol once daily, which was doubled every 2 weeks after confirming the absence of adverse drug reactions, such as clinically significant bradycardia, syncope, arterial hypotension or signs of pulmonary congestion. The maximal dosage of metoprolol in this study was 50 mg three times daily. At echocardiography a cannula was inserted into a left forearm vein. After 15 min in the supine position, basal recordings were taken. A continuous intravenous infusion of milrinone (Sanofi Winthrop GmbH, Munich, Germany) was started with 5 μg/kg body weight per min for 10 min and was increased to 7.5 and 10 μg/kg per min for 5 min each. Higher dosages were not applied to avoid cAMP-triggered arrhythmias. Echocardiographic and blood pressure recordings were taken at rest and 1 min before increasing the respective dose. At the beginning of the study and after 6 months, radionuclide ventriculography was performed at rest and under a moderate, submaximal workload of 50 W of bicycle exercise in the supine position. Healthy male volunteers underwent the same studies as the patients with heart failure. The effect of milrinone was studied under control conditions and after 150 mg of oral metoprolol within 10 h of echocardiography.

This study was in accordance with the declaration of Helsinki and was approved by the local ethics committee. Written informed consent was given by each patient.

Statistical analysis. The response to milrinone in the presence and absence of metoprolol was compared by two-way analysis of variance (ANOVA). Normal distribution of the sample was assessed by the Kolmogorov-Smirnov goodness of fit test. Probability values <0.05 were considered significant. Results are reported as mean value ± SD, unless otherwise indicated. Statistical analysis was performed using the SPSS for Windows package (version 7.0).

Results

Effects of milrinone on contractility. We set out to determine whether the effects of milrinone on contractility in vivo were independent of intact beta-adrenoceptors. Volunteers were studied under control conditions and after a cumulative metoprolol oral dose of 150 mg within 10 h of the investigation. Echocardiographic studies were performed during infusion of increasing concentrations of milrinone. The increase in fractional shortening was similar to that during application of the phosphodiesterase inhibitor under control conditions or after pretreatment with metoprolol (Fig. 1A). Similar results were obtained when the systolic P/D ratio was determined (control: 3.9 ± 0.4 to 4.8 ± 0.7 mm Hg/mm, $p < 0.05$; metoprolol: 3.5 ± 0.4 to 4.4 ± 0.9 mm Hg/mm, $p < 0.05$). The milrinone doses used did not significantly reduce mean arterial blood pressure and instantaneous resistance (not shown). In contrast, the effect of dobutamine was completely antagonized by treatment with metoprolol (Fig. 1B). Taken together, the data show that the effects of milrinone on contractility are independent of intact beta-adrenoceptors, because under these conditions the effect of dobutamine is markedly attenuated.

Effects of metoprolol in patients with heart failure. To investigate the effects of metoprolol treatment on milrinone effects on contractility, 15 patients with moderate heart failure were treated for 6 months with metoprolol (maximal dose

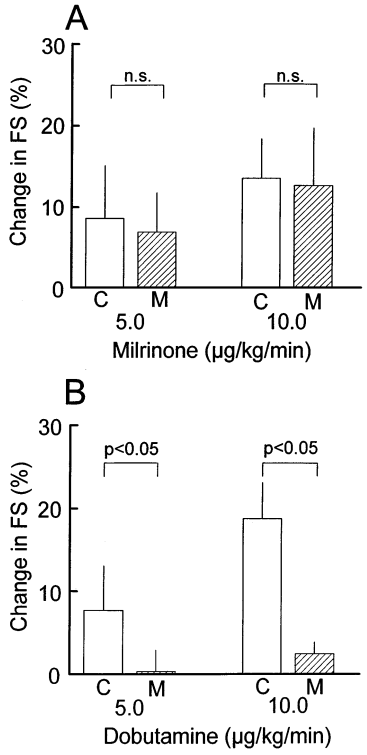


Figure 1. Change in fractional shortening (FS) under control conditions (C) or after treatment with metoprolol (M) (150 mg orally within 10 h of echocardiography) after application of milrinone **(A)** or dobutamine **(B)** in healthy volunteers.

50 mg three times daily). The clinical state of the patients improved from a mean functional class 2.9 ± 0.4 to 1.4 ± 0.5, and exercise tolerance changed from 62 ± 3 to 99 ± 5 W. Metoprolol treatment reduced heart rate from 91 ± 10 to 71 ± 9 beats/min and increased mean arterial blood pressure (mean ± SEM). In addition, left ventricular ejection fraction and fractional shortening were significantly increased. Neurohormonal activation, as judged from plasma norepinephrine concentrations, was reduced. Data are summarized in Table 1. To investigate whether metoprolol treatment affects the beta-adrenoceptor–independent effects of milrinone, dose-dependent effects were studied before and after 6 months of metoprolol treatment.

As shown in Figure 2, basal fractional shortening and P/D ratios were significantly increased after 6 months of metoprolol treatment. Before treatment with the beta-blocker, milrinone did not significantly increase fractional shortening (Fig. 2) or the P/D ratio (1.6 ± 0.3 vs. 1.6 ± 0.3 mm Hg/mm at 10 μg/kg per min, $p > 0.05$, two-way ANOVA). However, after 6 months of metoprolol treatment, fractional shortening at rest had increased significantly during intravenous application of milrinone (22.5 ± 1.9% to 29.0 ± 2.7%, $p < 0.05$, two-way ANOVA). Metoprolol treatment and short-term milrinone application were determined to be factors that each significantly influenced fractional shortening in an independent manner (two-way ANOVA). P/D ratio values were only slightly

Table 1. Effects of Six Months of Metoprolol Treatment

	Control	Metoprolol	p Value
LVEF (%)*	24.6 ± 1.5	40.3 ± 3.6	<0.01
FS (%)	13.2 ± 1.0	22.5 ± 1.9	<0.001
P/D (mm Hg/mm)	1.6 ± 0.3	2.1 ± 0.4	<0.0006
Ex tolerance (W)	62 ± 3	99 ± 5	<0.001
MAP (mm Hg)	86.5 ± 2.4	99.1 ± 5.2	<0.01
HR (beats/min)	91 ± 10	71 ± 9	<0.01
NYHA func class	2.9 ± 0.4	1.4 ± 0.5	<0.05
I	0	9	
II	2	6	
III	13	0	
NE (nmol/liter)	3.8 ± 0.5	2.7 ± 0.4	<0.05
BW (kg)	76 ± 15	83 ± 16	<0.05

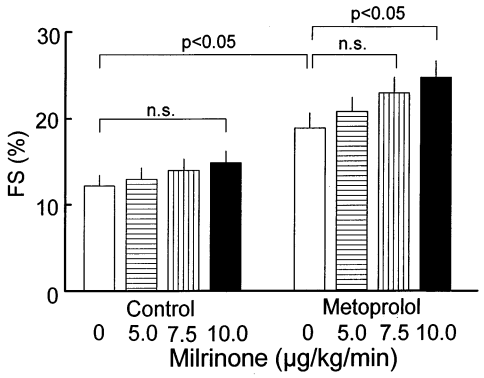
*Determined by radionuclide ventriculography. Data presented are mean value ± SD or number of patients. BW = body weight; Ex = exercise; FS = fractional shortening; func = functional; HR = heart rate; LVEF = left ventricular ejection fraction; MAP = mean arterial pressure; NE = norepinephrine; NYHA = New York Heart Association; P/D = pressure/dimension ratio.

increased by milrinone, from 2.1 ± 0.4 mm Hg/mm (control) to 2.4 ± 0.6 mm Hg/mm (p = NS) after metoprolol treatment. Taken together, long-term metoprolol treatment in patients with heart failure restores the cAMP-dependent inotropic effect of the cAMP–phosphodiesterase inhibitor milrinone, which is independent of beta-adrenoceptors.

Discussion

The present study investigated whether long-term beta-blocker therapy with metoprolol is capable of restoring the cAMP-dependent but beta-adrenoceptor–independent inotropic effects of milrinone in patients with heart failure. Metoprolol treatment for 6 months increased left ventricular ejection fraction, exercise tolerance and functional class. In addition, metoprolol reduced serum norepinephrine concentrations. These effects were accompanied by a restoration of the inotropic effects of milrinone, which were demonstrated to be independent of the intactness of beta-adrenoceptor signaling.

Figure 2. Fractional shortening (FS) after application of milrinone in patients with heart failure before (Control) or after 6 months of metoprolol treatment.



Treatment with metoprolol. Fifteen patients with dilated cardiomyopathy and moderate heart failure were treated according to an accelerated protocol of the Metoprolol in Dilated Cardiomyopathy (MDC) trial (13). Metoprolol produced a similar frequency reduction and a slightly more pronounced effect on left ventricular ejection fraction than that observed in the MDC trial. The mean metoprolol doses achieved in the present study were slightly higher than those achieved in the MDC trial, which could explain the slightly better effect on left ventricular ejection fraction. Taken together, the patient group studied and the effects of treatment closely resemble those of the MDC trial.

One important mechanism of contractile dysfunction in heart failure is the desensitization of beta-adrenergic signal transduction mechanisms (1–3). The number of beta-adrenoceptors has been reported to be reduced at the protein (1–3) and mRNA levels (22). The remaining receptors are presumably uncoupled (3) by an increase in activity and mRNA of the beta-adrenoceptor kinase (22).

Mechanisms of beta-adrenergic desensitization. These alterations produce a strong impairment of the positive inotropic effects of beta-adrenoceptor agonists in vitro (1–3) and in vivo (23). However, the positive inotropic effects of cAMP–phosphodiesterase inhibitors are also reported to be reduced (4,5).

As the mechanism for this reduced effectiveness, an increase in Giα on protein (6–8) and mRNA levels (24) has been reported by several groups. In pertussis toxin–treated membranes from failing hearts, the depressed basal and guanine nucleotide–stimulated adenylyl cyclase was reported to be restored by pertussis toxin, which inactivates Giα (7). In addition, in isolated cardiomyocytes from failing hearts, the effects of the beta-adrenoceptor agonist isoproterenol were restored to values similar to those observed in cardiomyocytes from nonfailing hearts (25). These studies provided strong biochemical and functional evidence for a significant contribution of Giα to the beta-adrenergic signal transduction defect in the failing heart by depressing basal cAMP formation (26). The reduction of the milrinone effects on force of contraction is closely correlated to the increase in Giα protein concentrations, as shown by in vitro studies (2). Finally, the positive inotropic effect of milrinone is restored when basal cAMP formation is enhanced by low concentrations of isoprenaline (27) or forskolin (5). Therefore, the reduced basal cAMP content due to elevated Giα is most likely responsible for the reduced effects of cAMP–phosphodiesterase inhibitors like milrinone. The other components of the adenylyl cyclase system, namely, the stimulatory G-protein alpha-subunits (Gsα), and the catalytic activity of adenylyl cyclase have been shown to be unchanged (2,7,28). Hitherto, it was unknown whether the increase in Giα could be pharmacologically modulated in humans.

Desensitization of beta-adrenergic signal transduction can be induced by treatment of cultured neonatal rat cardiomyocytes with norepinephrine in vitro (9). In these studies, the mechanism was also a decrease in beta-adrenoceptors and an increase in Giα. The effects could be antagonized by application of beta-blockers but not by the alpha-blocker prazosin, showing that the increase in Giα and beta-adrenoceptor downregulation is mediated by stimulation of beta-adrenoceptors (9). Müller et al. (29) consistently reported in nuclear runon assays an increase in the transcriptional activity of the Giα_2 gene in rats treated with isoprenaline. Thus, it is very likely that this adrenergic desensitization in the human failing heart is induced in general by the sympathetic alteration and in particular by the local norepinephrine release with consecutive beta-adrenoceptor stimulation in the failing heart.

Effects of beta-blocker treatment. From these studies on the pathophysiology of signal transduction in heart failure, it is tempting to speculate that beta-blockers, at least in part, can restore receptor and postreceptor defects. Heilbrunn et al. (15) reported that the increase in left ventricular ejection fraction after beta-blocker therapy with metoprolol for 6 months was accompanied by an increase in beta-adrenoceptor density, as judged from radioligand binding studies in myocardial biopsy specimens. In these patients, the inotropic effect of the beta-adrenoceptor agonist on the force of contraction, determined after discontinuation of beta-blocker therapy, was restored. In the present study, we addressed the question of whether postreceptor mechanisms, presumably the result of an increase in Giα content, are also restored by beta-blocker treatment. Determination of Giα content with immunochemical techniques is difficult in myocardial biopsy samples because larger amounts of tissue are required to obtain reliable results. Quantitative polymerase chain reaction is hampered by the great homology between the different G-protein subtypes. Therefore, we studied the effects of milrinone in vivo, which is clearly dependent on postreceptor mechanisms and independent of the function of beta-adrenoceptors. The restoration of inotropic responses to milrinone provides strong evidence that a postreceptor event involving adenylyl cyclase is favorably influenced by metoprolol treatment. Interestingly, the beta-adrenoceptor antagonist carvedilol increased left ventricular ejection fraction similar to metoprolol but had no effect on beta-adrenoceptor density; however, myocardial beta-adrenoceptor density was almost doubled after 6 months of metoprolol treatment in patients with heart failure (16). These data suggest that beta-blockers also target postreceptor events not just receptors. During the preparation of the present clinical study, a report appeared that investigated the amount of Giα-related pertussis toxin substrates in the myocardial biopsy specimens of patients before and after 3 months of metoprolol treatment (30). Treatment with metoprolol increased oxygen uptake at the anaerobic threshold, increased beta$_1$-adrenoceptors and reduced Giα-related pertussis toxin substrates (30). Therefore, the present observational data suggest that the reduction of Giα also has functional relevance for inotropic interventions. Consistent with the restoration of postreceptor alterations, reported to be induced by beta-adrenoceptor stimulation (10,29), the present study and previous investigations in patients with dilated cardiomyopathy (31) found reduced norepinephrine plasma concentrations after

beta-blocker treatment. In addition, the present findings could have important implications for the treatment of heart failure with cAMP–phosphodiesterase inhibitors. Clinical trials of milrinone have been negative for improved survival (32). A proarrhythmic potency and a positive chronotropic effect in the presence of a reduced inotropic efficacy have both been mentioned as a reason for the negative results. If concomitant beta-blocker treatment can prevent the former adverse effects (in partial or positive chronotropism) and can restore the inotropic efficacy of cAMP-phosphodiesterase inhibitors by reversing postreceptor alterations, combination therapy with cAMP–phosphodiesterase inhibitors and beta-blockers could be used to treat heart failure and would be worthwhile to investigate.

Conclusions. Metoprolol treatment restores cAMP-dependent positive inotropic effects independent of beta-adrenoceptors. This restoration most likely involves a modulation at the level of inhibitory G-proteins. These data suggest a new mechanism to explain the effect of beta-blockers on cardiac performance in patients with heart failure.

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